Spectroscopic Detection of Organophosphorus Agents

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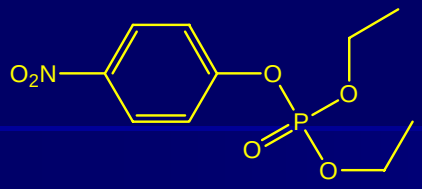
Insecticides and pesticides

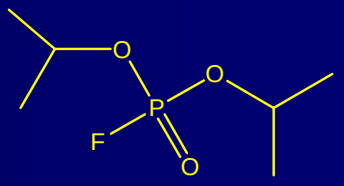


Diethyl p-nitrophenyl phosphate

(Paraoxon)

Diethyl para-nitrophenol thiophosphate

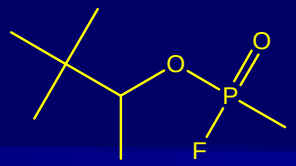
(Parathion)



Di-isopropyl fluoro-phosphate

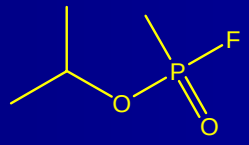
(DFP)

Nerve gases



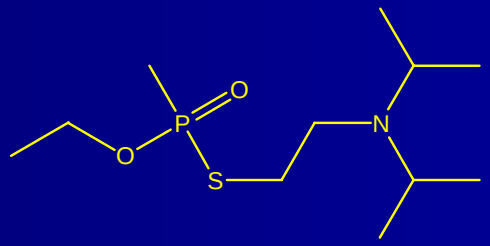
Phosphonofluoridic acid, methyl-, 1,2,2-trimethylpropyl ester

(Soman)



Methylphosphonofluoridic acid 1-methyl-ethyl ester

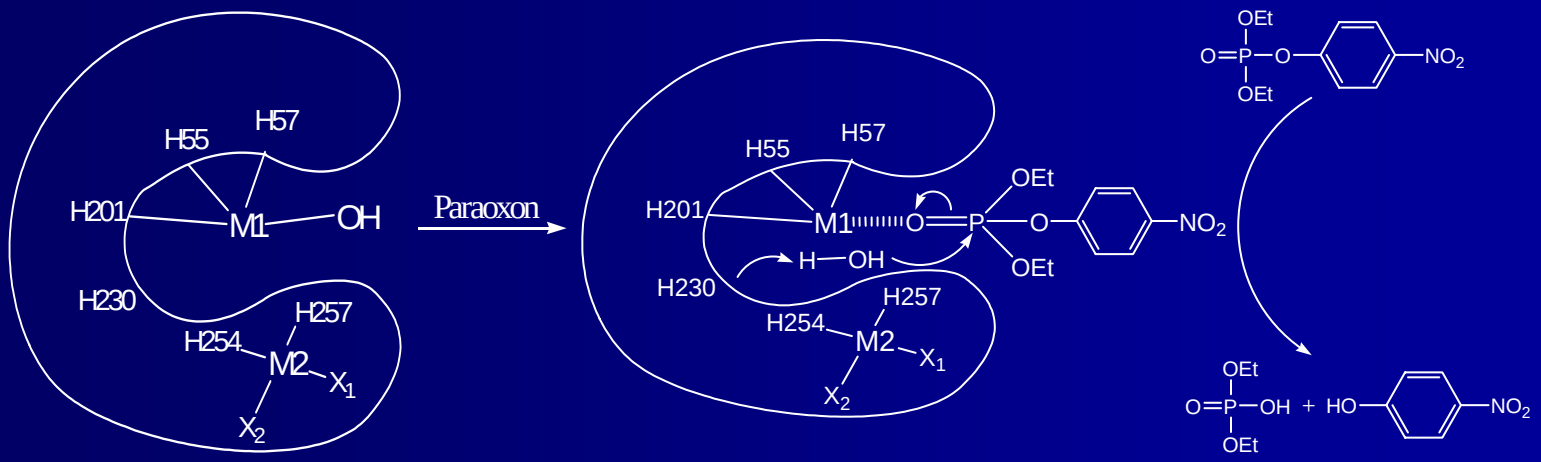
(Sarin)



O-Ethyl S-(2-(diisopropylamino)ethyl)methylphosphonothioate

(VX)

Organophosphorus Hydrolase



- Isolated from *Pseudomonas diminuta*
- Metalloenzyme (zinc at active site)
- S_N2 mechanism of hydrolysis

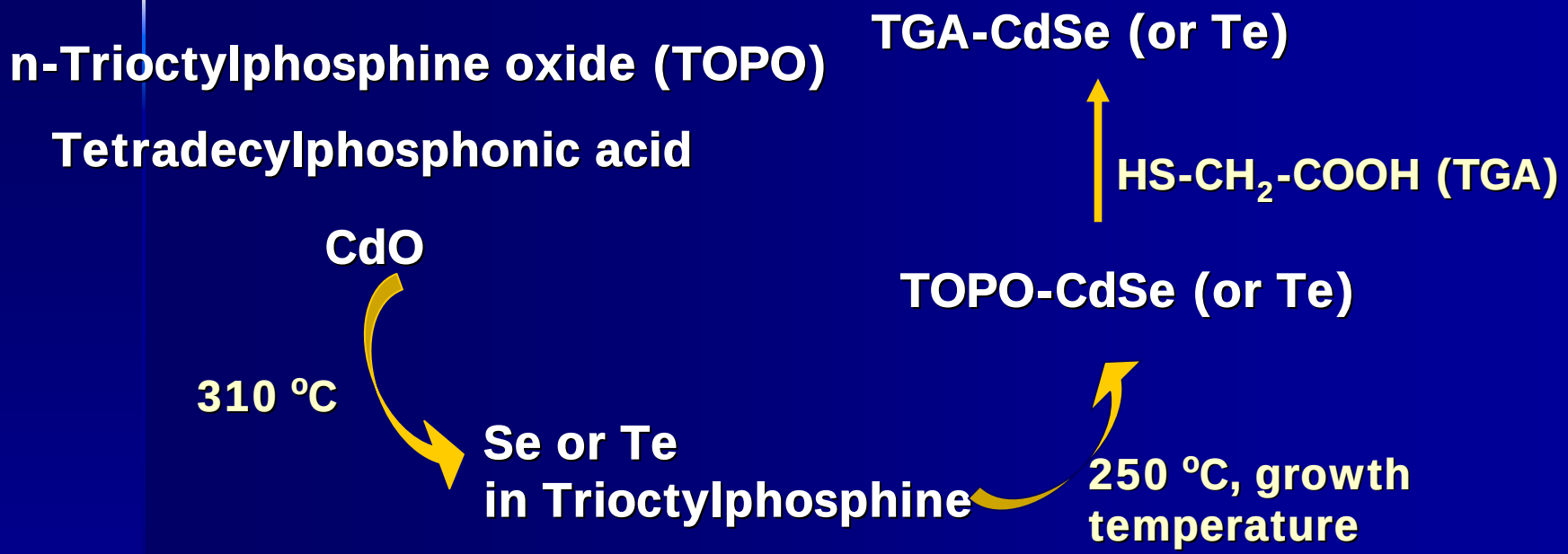
Quantum Dot Characteristics

- Quantum dots
  - Size dependent emission
  - High quantum yield
  - Broad excitation spectra
  - Narrow emission band
  - Chemical versatility
- Core-shell quantum dots
  - Higher quantum yield
  - Chemical and photophysical stability
  - Low toxicity

1.9 2.4 4.1 5.2 nm

Excited with same wavelength (365 nm)

Synthesis of Quantum Dots

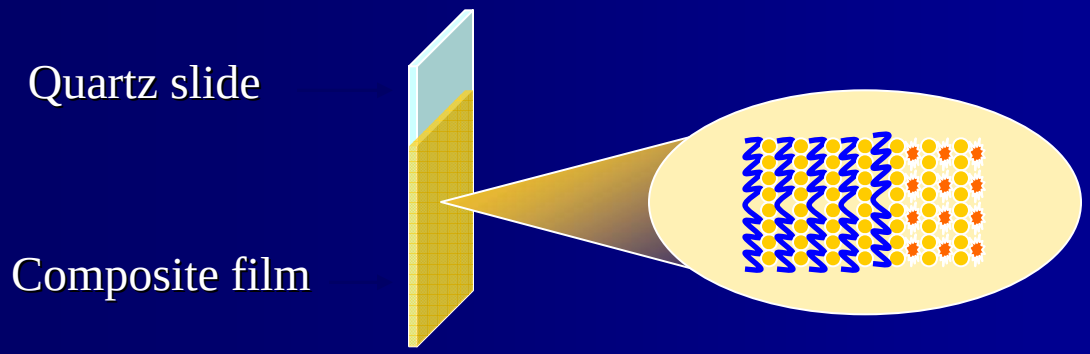


Adam-Peng, Z. and Peng, X.
J. Am. Chem. Soc. **2001**, 123, 183-184

Chan, W. C. W. and Nie, S.
Science **1998**, 281, 2016-2018

Sensing device 1: CdSe composite films : paraoxon detection

- ✓ Layer-by-Layer technique (electrostatic interaction)



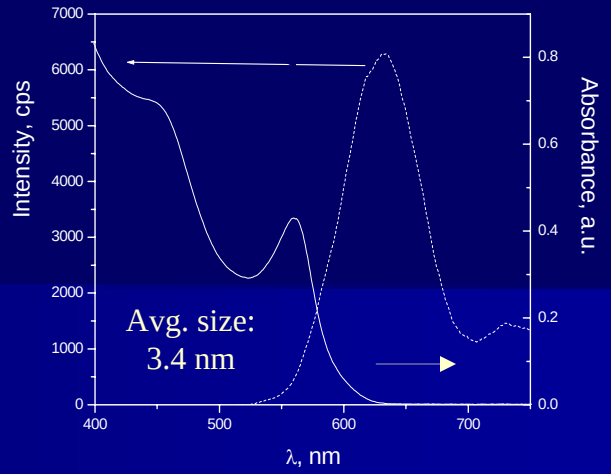
Chitosan

CdSe-S-CH_2-COOH quantum dots

Organophosphorus hydrolase (OPH)

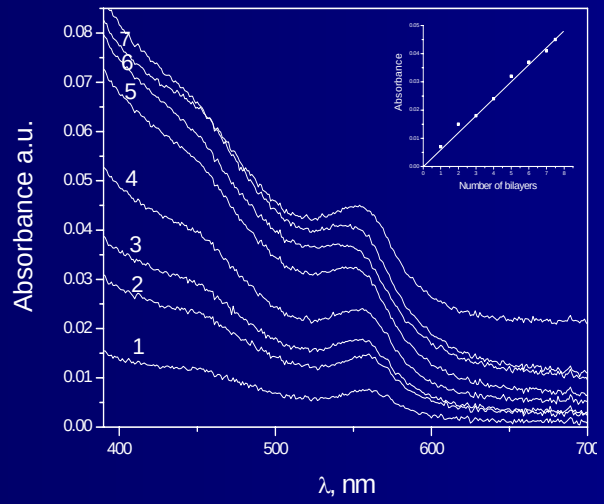
Constantine, C. A.; Gattás-Asfura, K. M.; Mello,S. V.; Crespo, G.; Rastogi,V.; Cheng, T.-C.; DeFrank, J. J. and Leblanc, R. M. *Langmuir* 2003, 19, 9863-9867

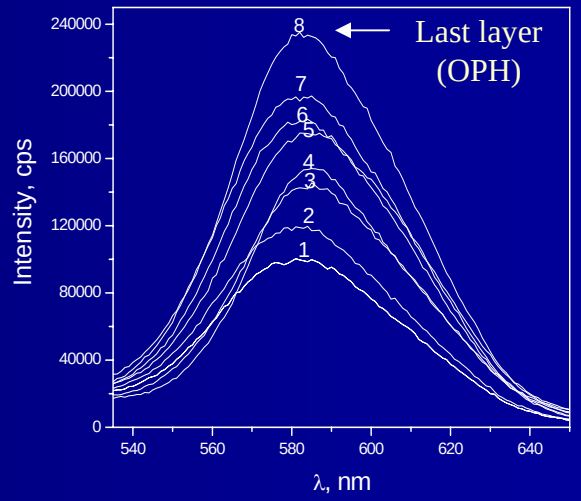
Optical properties of the quantum dots in aqueous solution :



Monitoring growth of the composite film on the quartz slide :

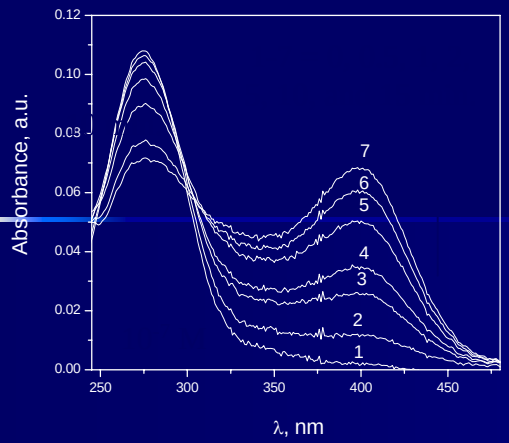
(1-7 = # layers of quantum dots)

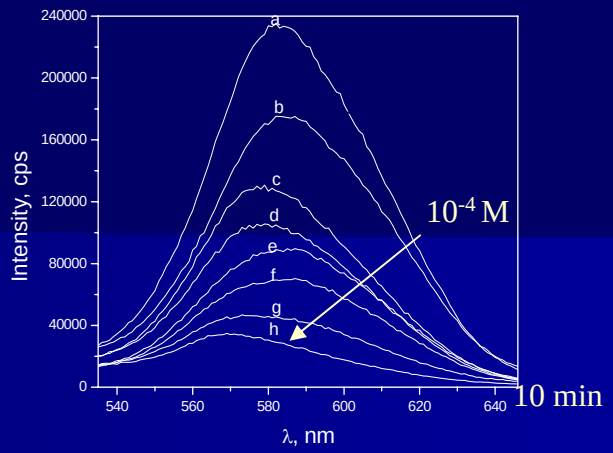


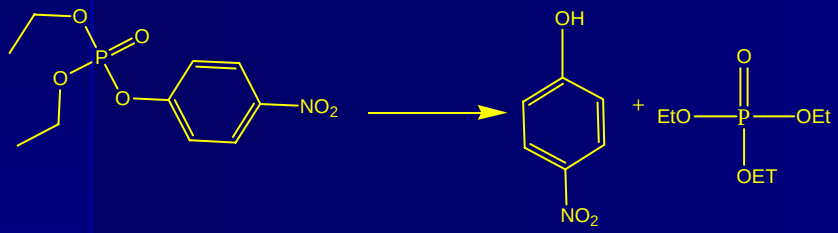


Constantine, C. A.; Gattás-Asfura, K. M.; Mello,S. V.; Crespo, G.; Rastogi,V.; Cheng, T.-C.; DeFrank, J. J. and Leblanc, R. M. *Langmuir* 2003, 19, 9863-9867

Response of film upon exposure to paraoxon solution







Epifluorescence images of film

895µm × 713 µm

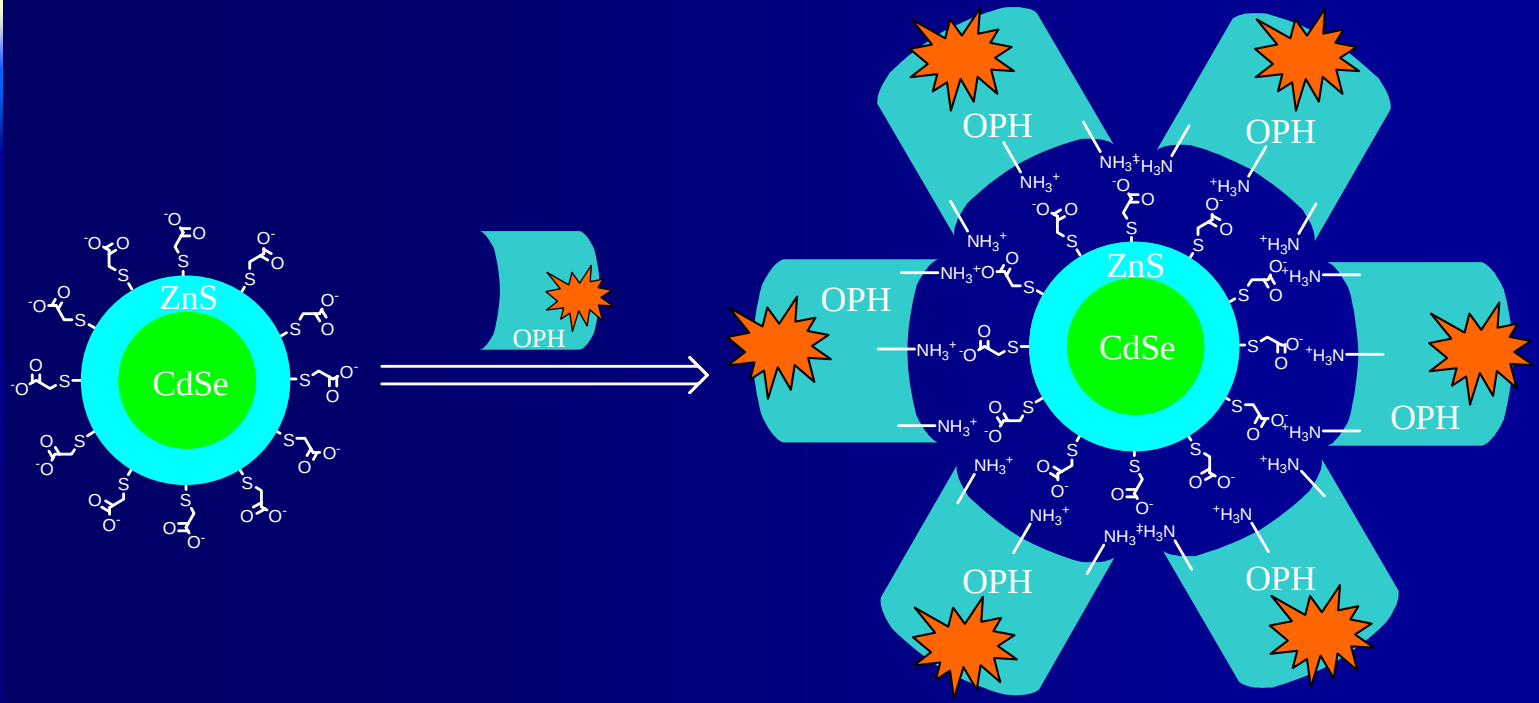
Constantine, C. A.; Gattás-Asfura, K. M.; Mello,S. V.; Crespo, G.; Rastogi,V.; Cheng, T.-C.; DeFrank, J. J. and Leblanc, R. M. *Langmuir* 2003, 19, 9863-9867

Conclusion

- Quantum dots were successfully immobilized into composite films through utilization of the Layer-by-Layer technique and electrostatic interactions

- The convenient quantum dot-OPH composite film fabricated was highly selective and sensitive towards organophosphorus compounds (e.g. paraoxon)

Sensing device 2: Formation of OPH/QDs bioconjugate



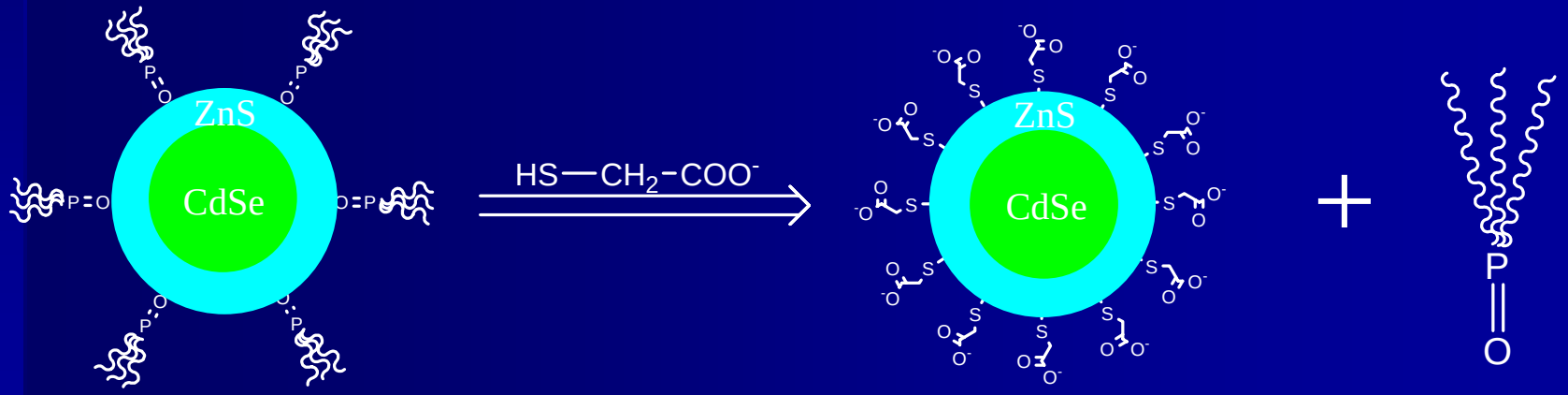
(= Active Site of OPH)

Formation of OPH/QDs bioconjugation

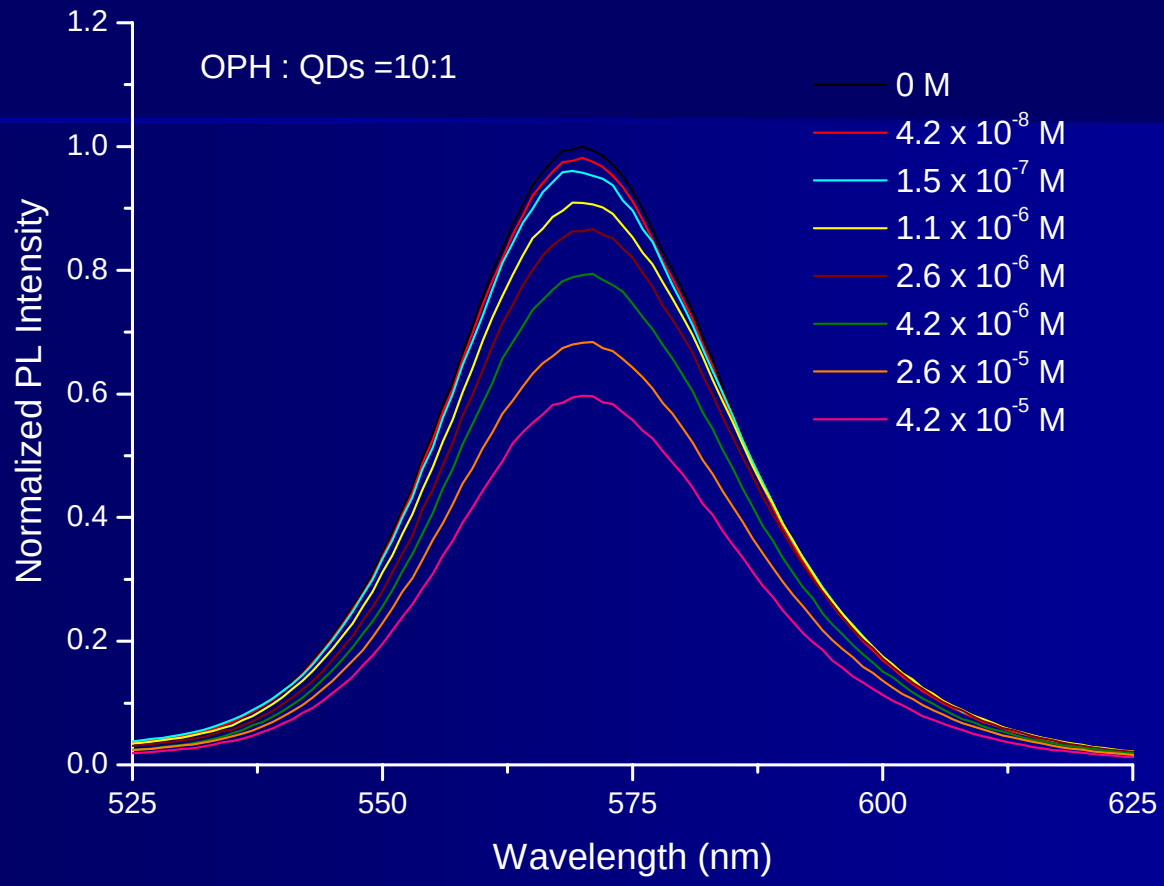
Synthesis of CdSe(ZnS) QDs

$$CdO + Se \xrightarrow[TOPO,\ 250\ ^oC]{TDPA,\ TOP} CdSe\ QDs$$

$$CdSe + (TMS)_2S + Zn(C_2H_5)_2 \longrightarrow CdSe(ZnS)\ QDs$$

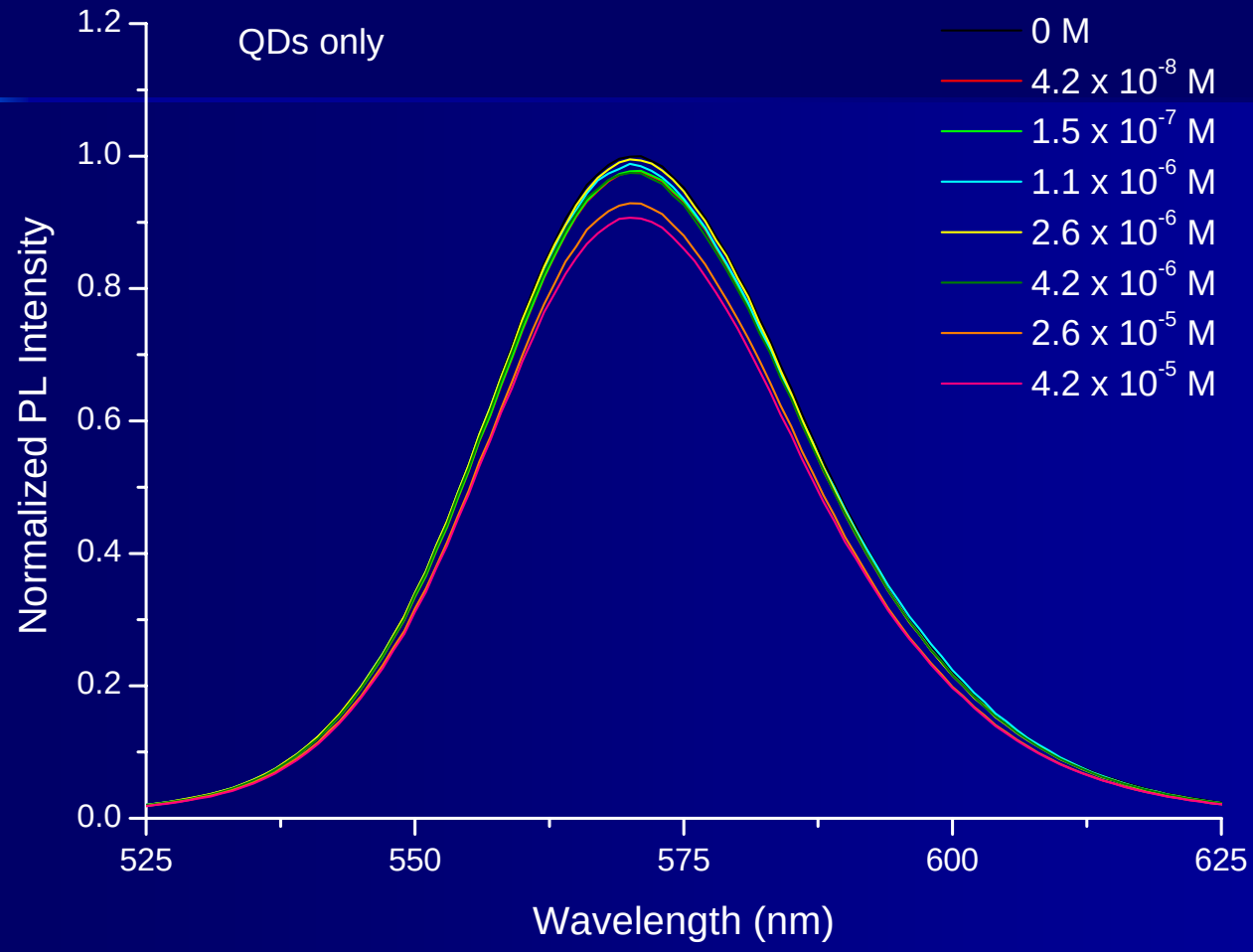


PL spectra of OPH/QDs



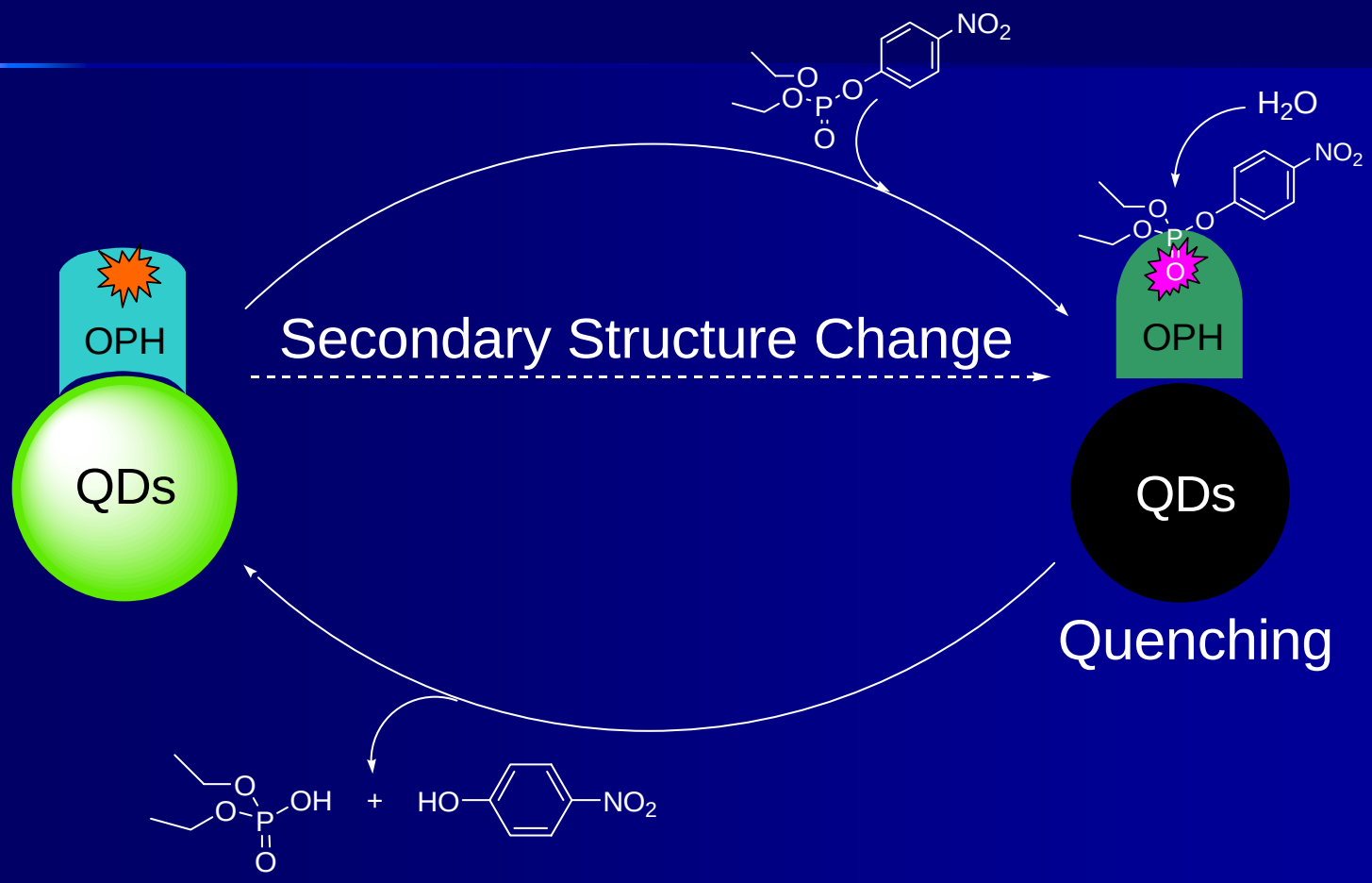
Photoluminescence spectra of 10:1 molar ratio OPH/QDs bioconjugate in different concentrations of paraoxon. Samples were excited at 350 nm.

PL spectra of pure QDs



Photoluminescence spectra of pure QDs in different concentrations of paraoxon. Samples were excited at 350 nm.

Mechanism of PL quenching



Michaelis-Menten Equation

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} E + P$$

$e_0 - [ES]$ $\quad$ $s_0 - [ES]$ $\quad$ $[ES]$

Steady state:

$$d[ES]/dt = k_{+1}(e_0 - [ES])[S] - k_{-1}[ES] - k_{+2}[ES] = 0 \quad ([S] = s_0 - [ES])$$

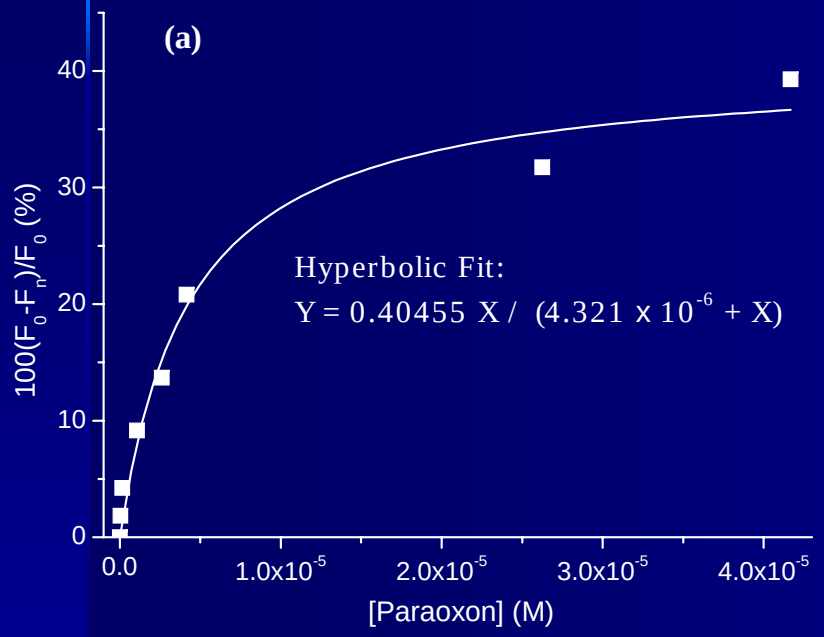
$$\Rightarrow \quad [ES] = \frac{k_{+1}\, e_0\, [S]}{k_{-1} + k_{+2} + k_{+1}[S]}$$

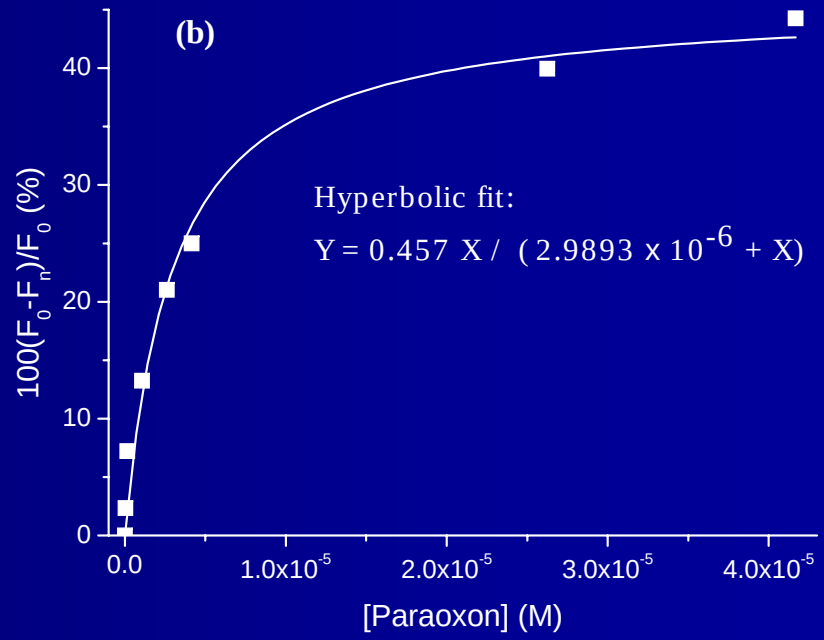
$$\Rightarrow \quad [ES] = \frac{e_0\, s_0}{(k_{-1} + k_{+2}) / k_{+1} + s_0} \qquad (s_0 >> e_0 > [ES])$$

$$\Rightarrow \quad Q = k_q[ES] = \frac{k_q e_0\, s_0}{(k_{-1} + k_{+2}) / k_{+1} + s_0}$$

Mechanism of PL quenching

Relative PL intensity percentage of (a) 10:1 (molar ratio) and (b) 100:1 (molar ratio) OPH/QDs bioconjugates as a function of paraoxon concentration.



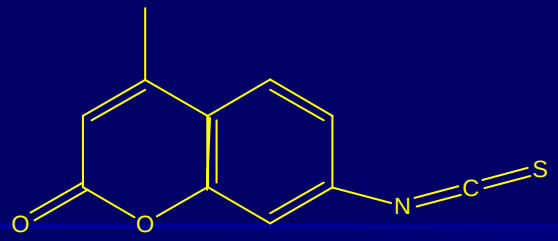


F_0: PL intensity at 573 nm in the absence of paraoxon
F_n: PL intensity at 573 nm in the presence of paraoxon

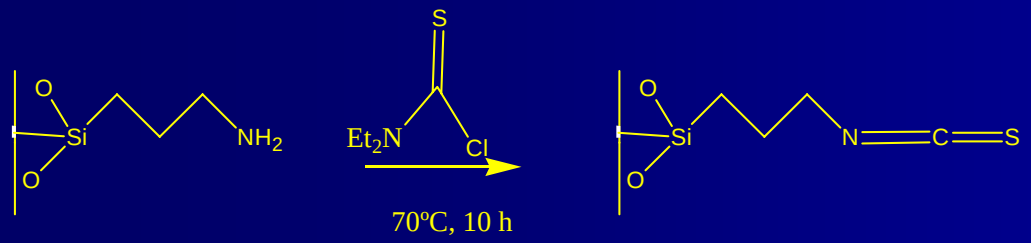
Conclusion

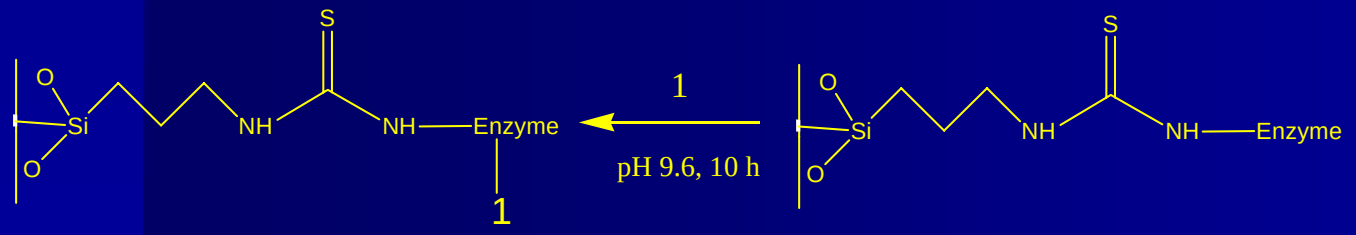
- The OPH and CdSe(ZnS) QDs can form stable bioconjugate through electrostatic interaction
- CD spectra indicate a secondary structure change of OPH in the presence of paraoxon
- The intensity of photoluminescence of OPH/QDs bioconjugate was quenched in the presence of paraoxon
- The secondary structure change of OPH was responsible for the observed PL quenching
- Increasing the molar ratio of OPH over QDs will not substantially increase the sensitivity of OPH/QDs biosensor

Sensing device 3: OPH covalently bound to solid support



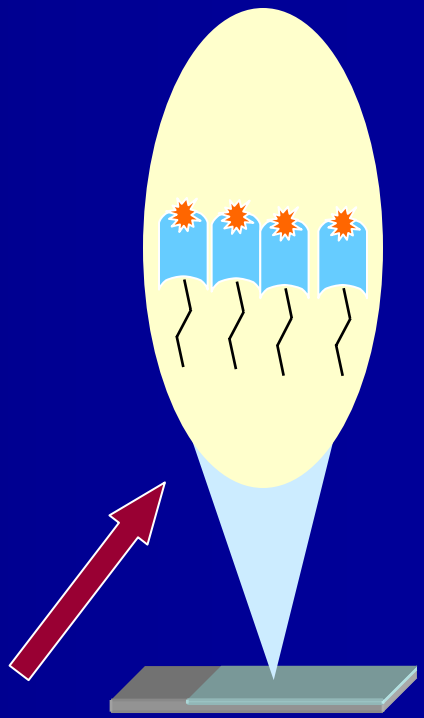
7-isothiocyanato-4-methylcoumarin (1)



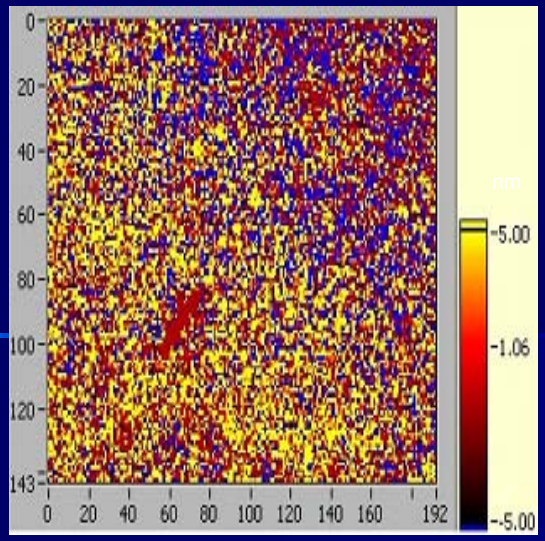


OPH

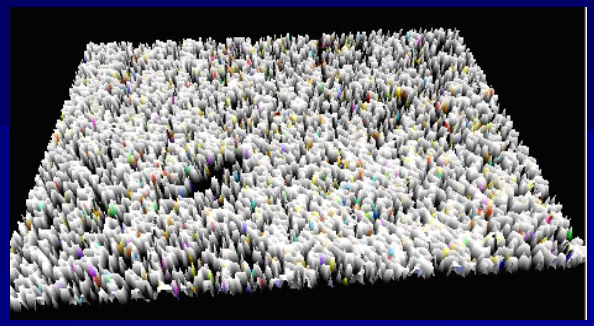
pH 9.6, 10 h



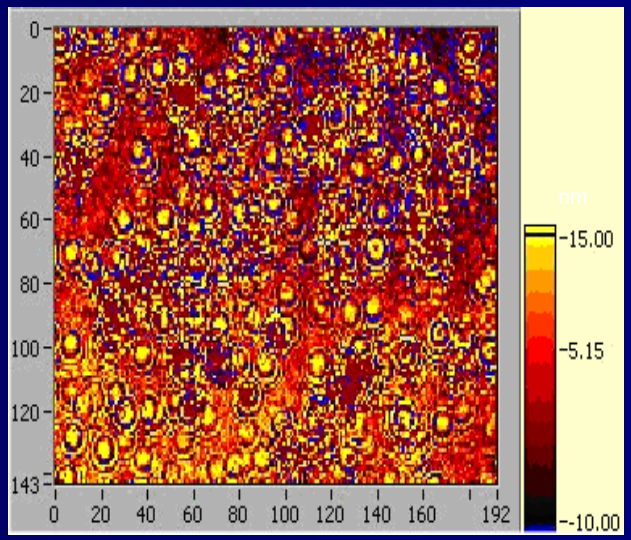
Imaging ellipsometry



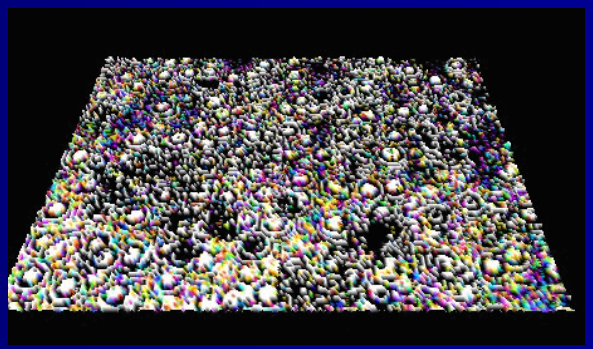
2-D image of the silanized slide



3-D image of the silanized slide

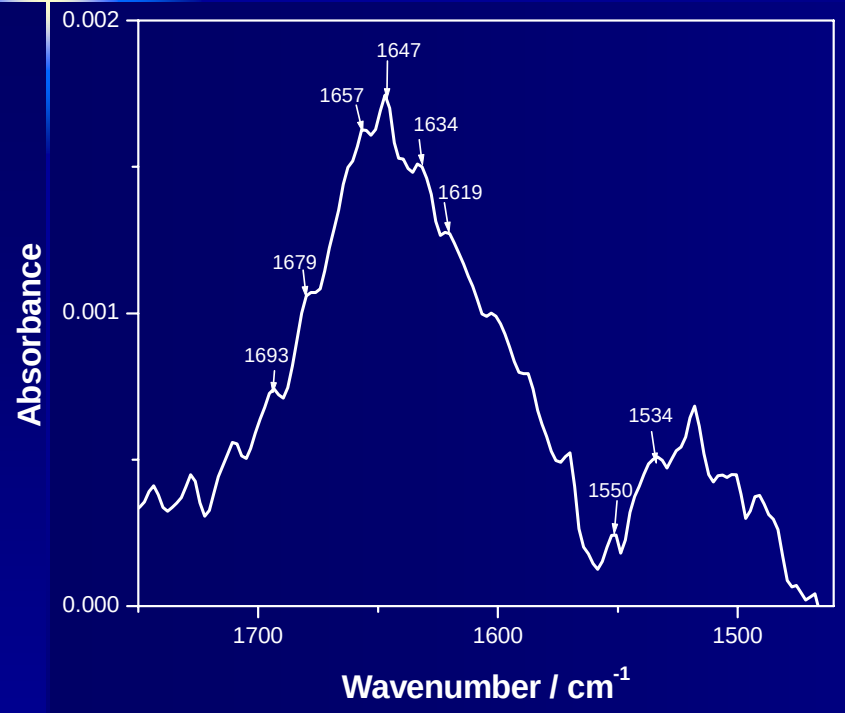


2-D image of covalently attached OPH



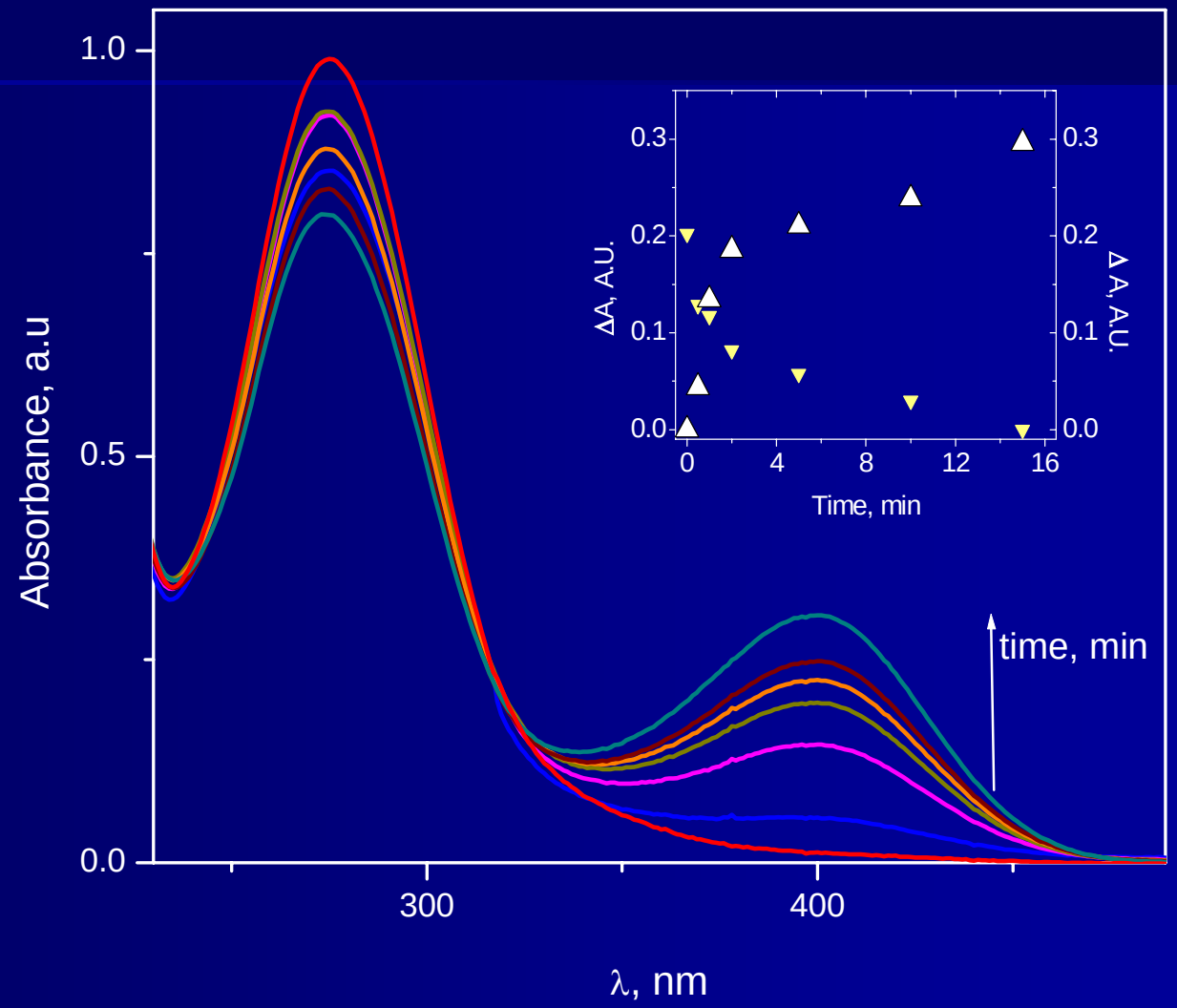
3-D image of covalently attached OPH

ATR-IR spectrum of the secondary structure of OPH

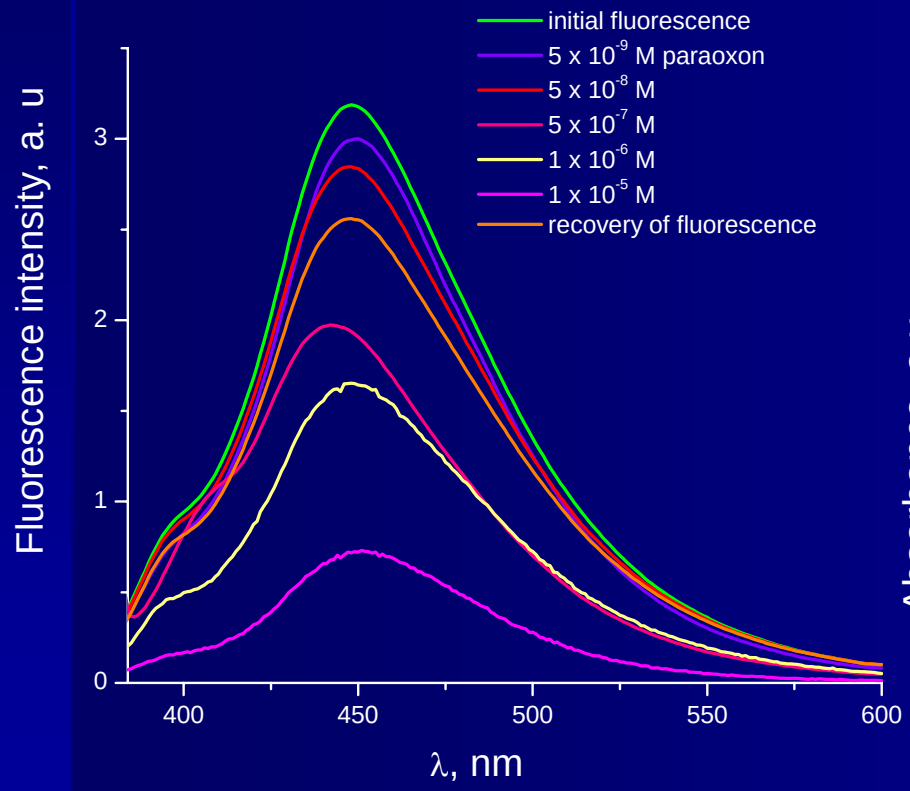


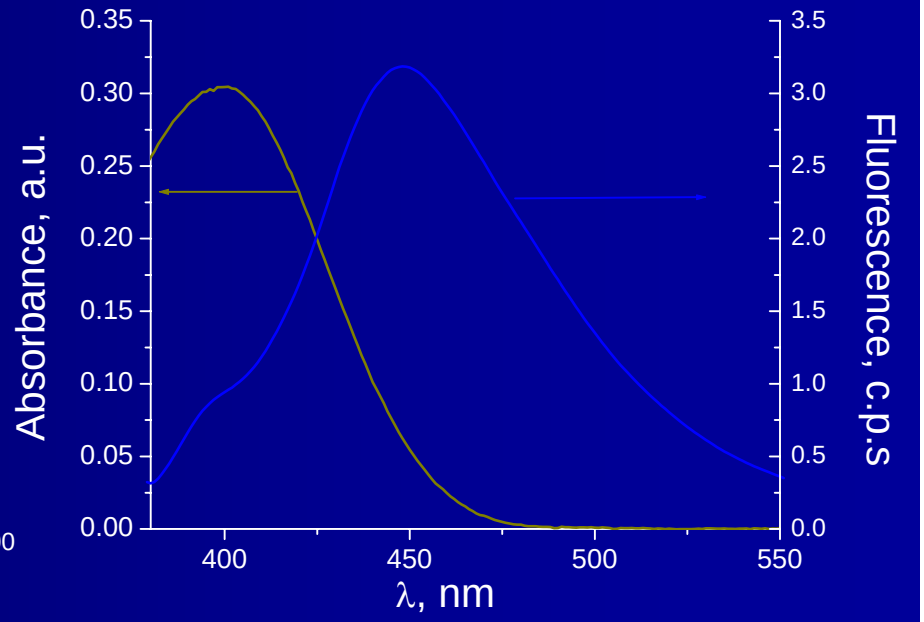
Frequency band position (cm^{-1})	Assignment
1693	anti-parallel ß-sheet or pleated turn
1679	ß-sheet
1657	α-helix
1647	amide I
1634	ß-sheet
1619	ß-sheet
1550	α-helix
1534	amide II

UV-Vis spectra for paraoxon analysis



Fluorescence spectra of OPH-coumarin *sensing system* in presence of paraoxon solution

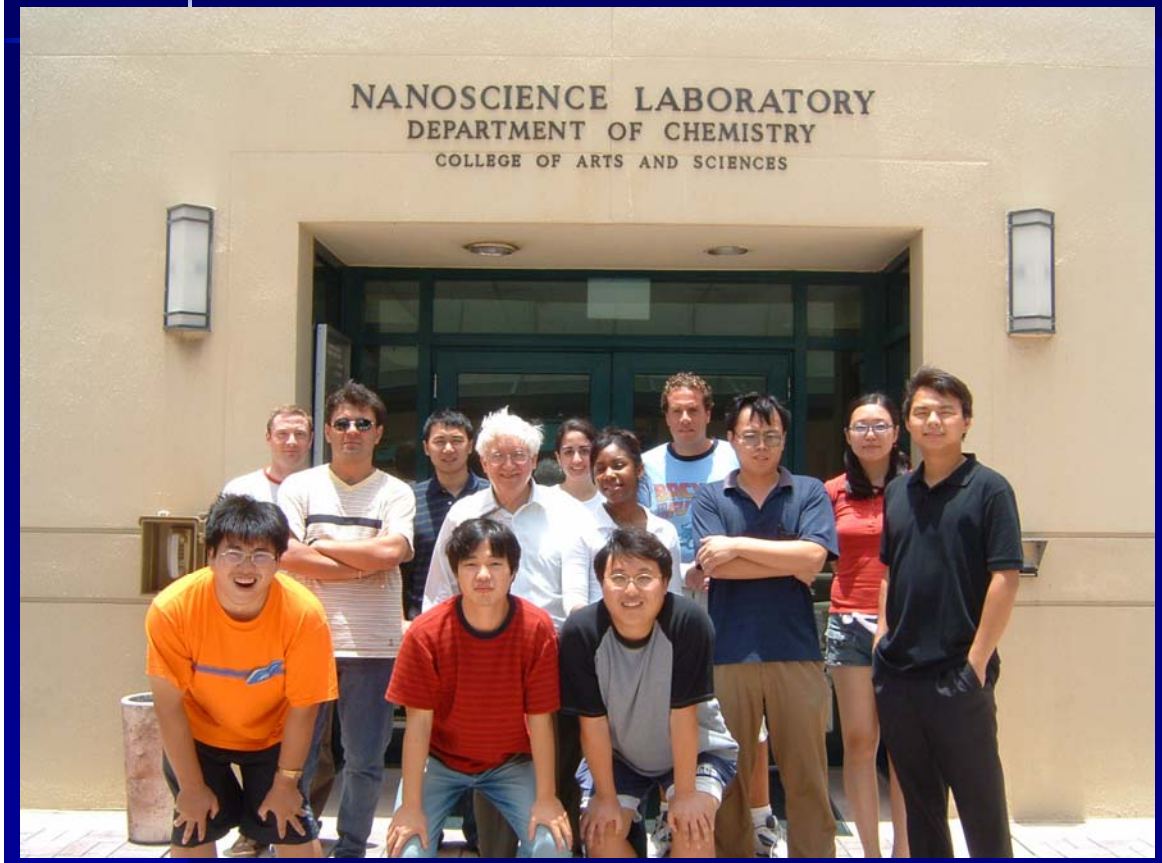




Conclusion

- OPH was successfully bound to a silanized quartz substrate
- This bio-functional surface successfully detects the presence of paraoxon aqueous solution

Group Members



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  - Michael J. Snedaker
  - Daniel A. Thimann
  - Sanjeev Sirpal
- Senior Research Assoc.
  - Jhony Orbulescu

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